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Comparison of L-tryptophan binding capacity of BSA captured by a polymer brush with that of BSA adsorbed onto a gel network

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Abstract

A polymer brush containing a diethylamino group as an anion-exchange group was appended onto a polymer substrate by radiation-induced graft polymerization and subsequent chemical modifications. Bovine serum albumin as a chiral ligand for L-tryptophan was bound to the polymer brush at a density ranging from 17 to 150 g BSA/l. For comparison, BSA was adsorbed onto the gel network containing a diethylaminoethyl group. The molar binding ratio of L-tryptophan to BSA on the polymer brush was 1.7-fold higher than that to BSA on the gel network.

Keywords: Polymer brush; Gel network; L-Tryptophan; Bovine serum albumin

1. Introduction

Polymer brush is defined as polymer chains grafted onto a substrate. Polymer brush charged via the introduction of ion-exchange groups, i.e. charged polymer brush, can expand from the substrate due to mutual electrostatic repulsion to hold proteins in a multilayer [1]. Muller [2] grafted an ion-exchange-group-containing polymer chain onto a polymeric bead, and demonstrated that proteins are bound in the multilayer. Multilayer protein binding, which was termed as tentacle binding, effectively increases the binding capacity for protein purification in pharmaceutical and food industries. Tsuneda et al. [3] and Matoba et al. [4] prepared a porous hollow-fiber membrane with graft chains containing diethylamino groups ($-N(C_2H_5)_2$) as an anion-exchange group. The graft chains captured bovine serum albumin and urease in the multilayer during permeation of the protein solution through the porous membrane. Similarly, the graft chains containing sulfonic acid groups ($-SO_3H$) as a cation-exchange group held lysozyme in the multilayer [5].

A membrane that immobilized SO_3H group-containing graft chains was applicable as an acid catalyst for hydrolysis of sucrose [6]. The graft chain exhibited catalytic activity comparable to sulfuric acid in solution for the hydrolysis. This demonstrates that the expanding graft chains have an activity level as high as that of homogeneous catalysts. The charged polymer brush will provide an unrestricted field for

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immobilization of biomolecules. Conformational distortion of the protein molecules captured by the graft chains can be minimized because the graft chains can hold the protein molecules in accordance with the conformation.

The conformation of proteins adsorbed on solid surfaces governs their functions such as molecular recognition and enzymatic activity. Kondo and Urabe [7] measured the circular dichroism spectra of α-amylases adsorbed on ultrafine silica particles to discuss the conformational changes accompanying the adsorption. In this study, we compare the chiral recognition performance of bovine serum albumin (BSA) captured by the anion-exchange-group-containing polymer brush with that of BSA adsorbed onto the anion-exchange gel network, by determining the binding capacity of L-tryptophan to BSA. BSA is reported to have specific binding sites for biomolecules such as L-tryptophan and L-kynurenine [8–15].

2. Experimental

2.1. Materials

Porous hollow-fiber membranes made of polyethylene with two different pore sizes were supplied by Asahi Kasei Corporation (Tokyo, Japan), and used as a trunk polymer for grafting. The inner and outer diameters of both hollow fibers were 1.8 and 3.1 mm, respectively. These hollow fibers had a three-dimensional pore network with a porosity of 70% and pore diameters of 0.4 and 0.5 μm. The former and latter hollow-fiber membranes are referred to as M and L fibers, respectively.

Glycidyl methacrylate (GMA, CH_2=CCH_3-$COOCH_2CHOCH_2$) was acquired from Tokyo Chemical (Tokyo, Japan), and was used without further purification. Bovine serum albumin (BSA), purchased from Sigma (A-7030) (St. Louis, MO, USA), was dissolved in 20 m*M* Tris–HCl buffer (pH 8.0). L-Tryptophan (L-Trp) was obtained from Nacalai Tesque (Kyoto, Japan). Other reagents were of analytical grade or higher.

Agarose-based gel beads containing diethylaminoethyl (DEAE) groups as an anion-exchange group, DEAE Sepharose FF, were purchased from Pharmacia Biotech (Uppsala, Sweden). The bead diameter ranged from 45 to 165 μm. These gel beads are referred to as DEAE beads.

2.2. Preparation of an anion-exchange porous membrane

An anion-exchange porous membrane was prepared by the following four steps (Fig. 1): (1) Electron beam irradiation of the trunk polymer. The porous hollow-fiber membrane made of polyethylene was irradiated with an electron beam in a nitrogen atmosphere at ambient temperature to produce radicals on the membrane. The dose was set at 200 kGy. (2) Grafting of GMA as a precursor monomer. The irradiated membrane was immersed in 10% (v/v) GMA–methanol solution that had previously been deaerated. The reaction was performed at 313 K. (3) Conversion of some epoxy groups to diethylamino (DEA) groups. The GMA-grafted membrane was immersed in a mixture of diethylamine ($NH(C_2H_5)_2$)–water (50:50, v/v) at 303 K. (4) Hydrophilization of the remaining epoxy groups. The membrane was soaked in ethanolamine ($NH_2C_2H_5OH$) at 303 K for 6 h. The preparation conditions are similar to those reported by Tsuneda et al. [16].

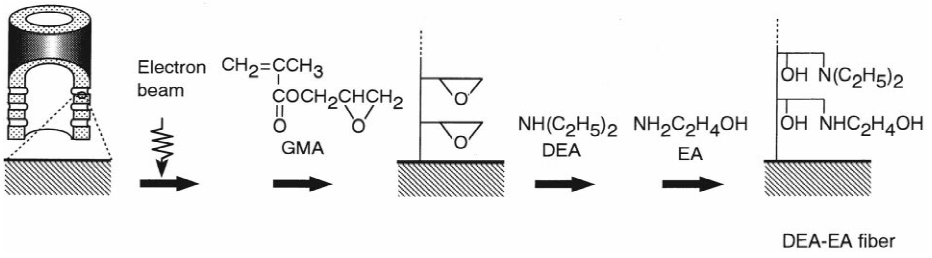

Fig. 1. Preparation scheme of an anion-exchange porous membrane for BSA immobilization.

The resultant porous hollow-fiber membrane containing both DEA and 2-hydroxyethylamino (EA) groups is referred to as DEA-EA(dg, x)-M or -L fiber, where dg and x are the degree of grafting and the conversion to DEA groups, respectively, and are defined as:

$$\text{Degree of grafting (dg, \%)} = \frac{100(\text{Weight increase due to grafting})}{(\text{Weight of the trunk polymer})} \quad (1)$$

$$\text{Conversion (x, \%)} = \frac{100(\text{Moles of the DEA groups})}{(\text{Moles of the epoxy groups before the addition reaction})} \quad (2)$$

Here, dg was set at 200 and 240% for M and L fibers, respectively. Moles of the DEA group were determined from the amounts of HCl adsorbed onto the DEA group: after the immersion of the hollow fiber in 0.2 *M* HCl, the decrease in HCl concentration determined by titration with 0.1 *M* NaOH was converted to the amount of HCl adsorbed onto the DEA group of the polymer brush [17].

2.3. Immobilization of BSA to a porous membrane and a gel bead

The DEA-EA(dg, x) fiber of 70 mm effective length was positioned in an I-configuration and the bottom end was plugged, as shown in Fig. 2a. A 2.0-g/l BSA buffer solution (pH 8.0) was applied in the interior of the hollow-fiber membrane and permeated through the pores radially outward under a constant permeation pressure of 0.02 or 0.03 MPa. The effluent penetrating the outside surface of the fiber was continuously sampled and its BSA concentration was determined by measuring the UV absorbance at 280 nm. Equilibrium binding capacity (EBC) of BSA for the feed solution was calculated as:

$$\text{EBC (g/l)} = \int_0^{V_e} (C_0 - C)\, dV/V_w \quad (3)$$

where C_0 and C are the BSA concentrations of the feed and effluent, respectively. V and V_e are the effluent volume and the effluent volume when C is equal to C_0, respectively. V_w is the volume of the membrane in a wet state calculated as:

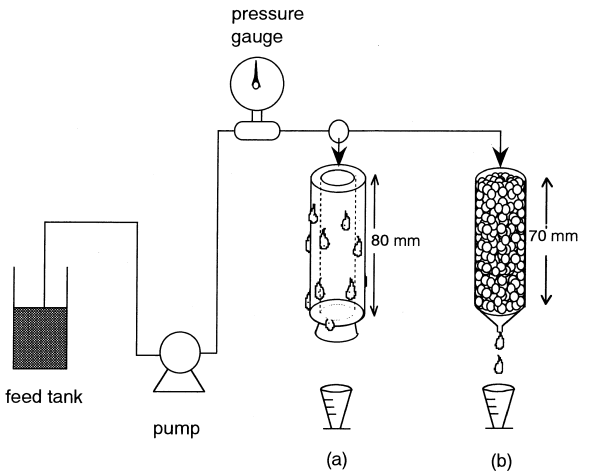

Fig. 2. Experimental apparatus for immobilization of BSA and binding of L-tryptophan. (a) Permeation through the hollow fiber. (b) Flow through the bead-packed column.

$$V_w = \pi(d_0^2 - d_i^2)\, L/4 \quad (4)$$

where d_i, d_0, and L are the inner and outer diameters, and length of the hollow-fiber membrane, respectively. The resultant membrane is referred to as a BSA(q)-M or -L fiber, where q in the parenthesis denotes the EBC of BSA.

In contrast, a prescribed amount of DEAE beads was immersed and gently stirred in 2.0 g/l BSA buffer solution (pH 8.0) for various immersion times. The resultant bead is referred to as a BSA(q) bead. The BSA bead was charged to the glass column of 4.7 mm inner diameter (d_c). The effective column length (L_c) was fixed at about 70 mm. The amount of BSA adsorbed on the column was measured after washing with a buffer and elution with a buffer containing 0.05 *M* NaCl, where the column volume (V_c) is defined as $\pi d_c^2 L_c/4$, where d_c and L_c are the inner diameter and length of the column, respectively. The experiments were performed at ambient temperature.

2.4. Binding of L-tryptophan to BSA immobilized on a graft chain and on a gel bead

The BSA(q) fiber of 70 mm effective length was

positioned in an I-configuration. Then, 6.0×10^{-5} *M* L-Trp in 20 m*M* Tris–HCl buffer (pH 8.0) was permeated through the pores from the interior of the hollow fiber to the exterior at a constant permeation pressure ranging from 0.01 to 0.04 MPa at 297 K (Fig. 2a). Similarly, the L-Trp solution flowed downward through the BSA(q) bead column at a constant operating pressure of 0.07 MPa (Fig. 2b). The L-Trp concentration of the effluent penetrating the outside surface of the hollow fiber or flowing out from the column exit was continuously determined by the measurement of UV absorbance at 280 nm. L-Trp concentration changes in the effluent were plotted against the effluent volume to obtain the breakthrough curves.

3. Results and discussion

3.1. Properties of anion-exchange porous membrane

The weights of the poly-GMA graft chains, i.e. the epoxy-group-containing graft polymer, were set at values 2.0- and 2.4-fold that of the M and L fibers, respectively. The epoxy group was converted to the diethylamino (DEA) group at a mole percentage of up to 96 and 98% for the M and L fibers, respectively, by varying the reaction time up to 24 h. The DEA group density ranged up to 3.6 mol per kg of the anion-exchange hollow-fiber membrane in a dry state. Volume expansion was observed during graft polymerization of GMA and subsequent introduction of the DEA group; the thickness of the DEA-EA hollow-fiber membrane ranged from 0.80 to 0.90 mm, which corresponded to an increase of 23–38% compared to the initial thickness (0.65 mm). The apparent density of the membrane, which is obtained by dividing the weight of dry membrane by the volume of wet membrane, was approximately 0.4 kg/l. A comparison of the properties between the DEA-EA(200, x)-M fiber and DEAE bead is summarized in Table 1.

3.2. Properties of BSA-immobilized porous membrane and gel bead

An example of the breakthrough curve of the DEA-EA(200, 96)-M fiber for BSA permeation is shown in Fig. 3. The abscissa is the dimensionless effluent volume (DEV) defined by dividing the effluent volume by the membrane volume including the lumen part, whereas the ordinate is the dimensionless concentration defined as the BSA concentration ratio of the effluent to the feed. BSA was quantitatively captured at an initial stage. After the breakthrough point, i.e. $C/C_0 = 0.1$, at DEV of approximately 50, BSA concentration increased gradually, and an equilibrium was attained at a DEV of approximately 150. Equilibrium binding capacity (EBC) of the fiber for BSA was calculated to be 150 g/l from Eq. (3).

Table 1
Comparison of the properties between the modified fiber and bead

	DEA-EA (200, x)-M fiber	DEAE bead
Matrix	Polyethylene	Cross-linked agarose
Anion-exchange group	Diethylamino (DEA) and 2-hydroxyethylamino (EA) groups	Diethylaminoethyl (DEAE) group
Size (mm)	Inner diameter 2.4 Outer diameter 4.2	Particle size range 0.045–0.165
Density of anion-exchange group (mol/l)[a]	DEA: 0.28–1.4	0.11–0.16
Binding capacity of BSA (g/l)[a]	17–150	21–100

[a] In a wet state.

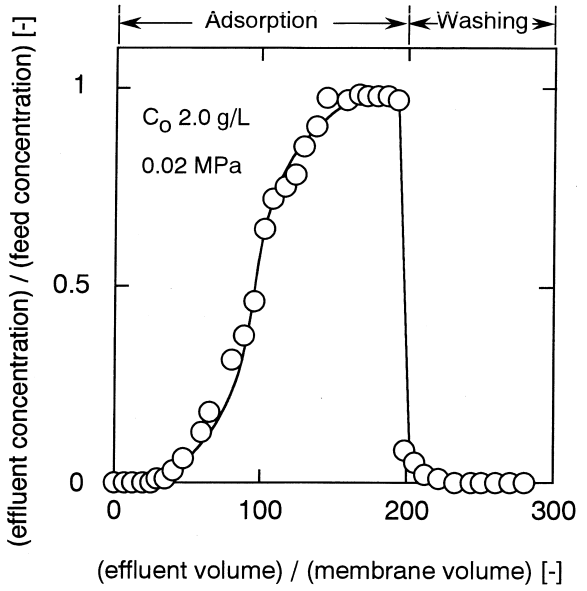

Fig. 3. An example of breakthrough curve of the DEA-EA(200, 96)-M fiber for BSA.

EBC of the fiber ranged from 17 to 150 g/l by adjusting the DEA group density of the fiber. Whereas, the amount of BSA adsorbed onto the DEAE bead for BSA ranged from 21 to 100 g/l by varying the immersion time in the BSA solution in a batch mode.

The space velocity (SV), defined by dividing the flow-rate by the membrane volume, of the BSA(65)-M and BSA(140)-L fibers is shown in Fig. 4 as a function of operating pressure, i.e. permeation pressure. The SV of the column charged with the BSA(100) beads, defined by dividing the flow-rate by the column volume, is also shown in Fig. 4. Space velocity represents the throughput for the practical use of both the hollow-fiber membrane and the column. In both the hollow fiber and the column, the SV increases linearly with increasing operating pressure; the compaction caused by the pressure was not observed in this flow-rate range. For identical volumes of the fiber and the column, the BSA-M and -L fibers exhibited 5- and 11-fold higher flow-rates, respectively, compared to the column at the same operating pressure. These flow characteristics of the BSA fibers are advantageous in that the fiber enables a high throughput of target molecules.

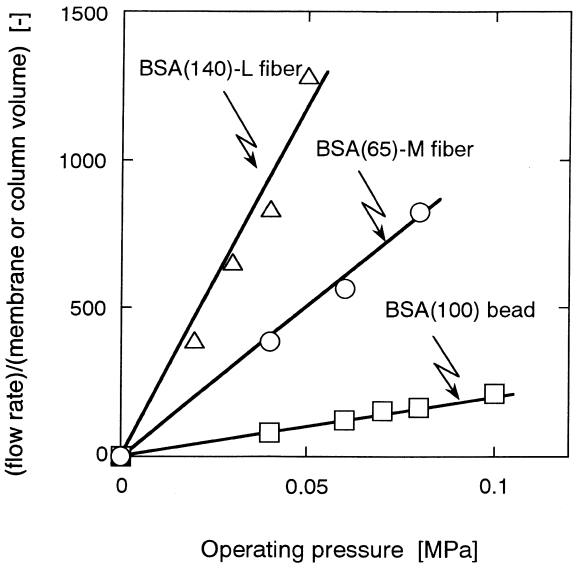

Fig. 4. Comparison between space velocities of BSA fibers and BSA-bead-packed column.

3.3. Comparison of L-tryptophan binding capacity

Breakthrough curves of the BSA(130)-L fiber for L-tryptophan (L-Trp) solutions are shown in Fig. 5

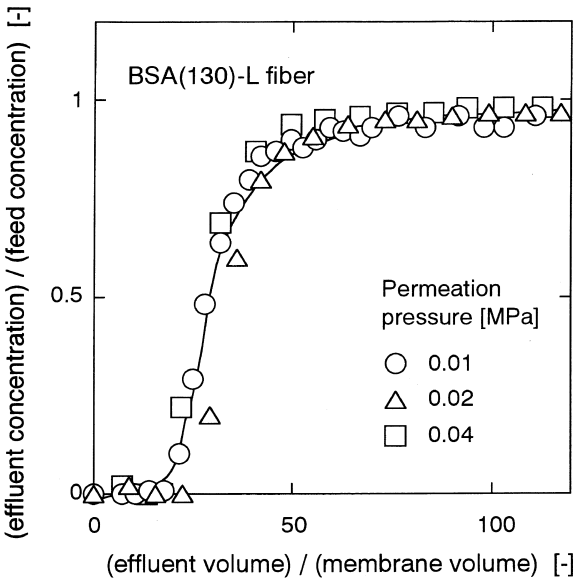

Fig. 5. Breakthrough curves of the BSA(130)-L fiber for L-Trp as a function of permeation pressure.

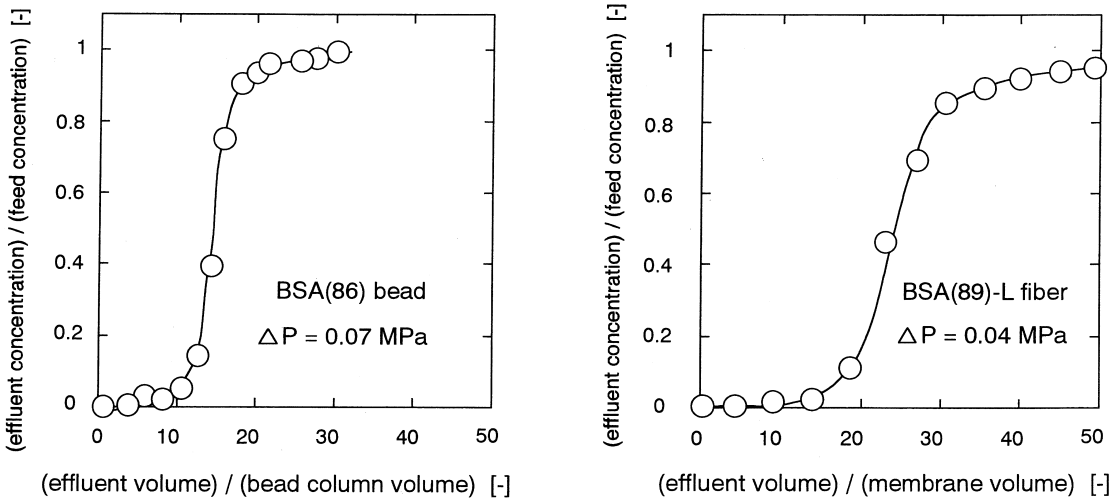

Fig. 6. Breakthrough curves of BSA fiber and BSA-bead-packed column for L-Trp.

for different permeation pressures. Irrespective of permeation pressure, i.e. residence time of the L-Trp solution through the pores across the fiber, the curves are seen to overlap. This indicates that the diffusional mass-transfer resistance of L-Trp to BSA immobilized on the graft chains is negligible because of the convective flow through the pores.

Breakthrough curves of the BSA(89)-L fiber and BSA(86) bead for L-tryptophan are compared in Fig. 6. The number of moles of L-Trp adsorbed onto the BSA was calculated by integrating the breakthrough curve. The molar binding ratio of the L-Trp to BSA immobilized onto the graft chain, i.e. polymer brush, is compared to that onto the bead, i.e. gel network (Fig. 7). Irrespective of the amount of BSA adsorbed, the ratio was constant at 0.82 and 0.49 for BSA bound to the brush and the network, respectively. The fact that BSA bound to the polymer brush exhibits a high molar binding ratio of BSA to L-Trp has been demonstrated.

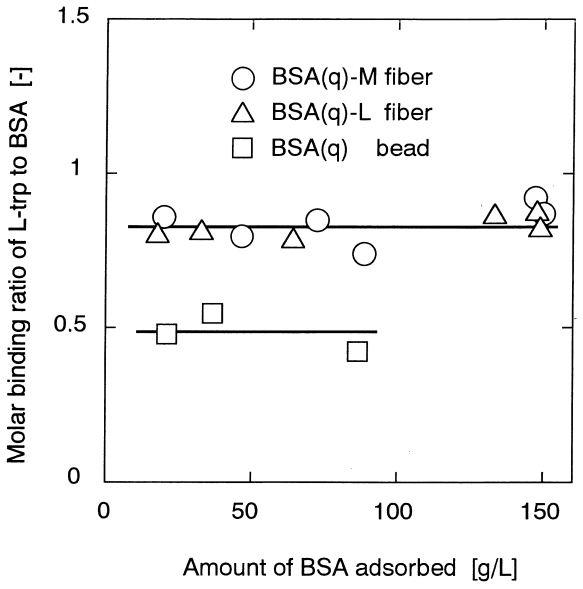

Fig. 7. Comparison of L-Trp molar binding ratio of BSA captured by the graft chain with that of BSA adsorbed onto the gel.

4. Conclusion

The polymer brush containing a diethylamino group as an anion-exchange group was appended onto a porous polymer substrate. Bovine serum albumin (BSA) immobilized to the charged polymer brush was applied to recognize L-tryptophan (L-Trp). Molar binding ratios of L-Trp to BSA captured by the polymer brush and BSA adsorbed onto a gel network were compared. High ratio of the former by

1.7-fold shows that the charged polymer brush effectively immobilizes BSA for recognizing L-Trp.

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